

# EFFICIENT GENERATION OF ADENOVIRUS-BASED LIBRARIES BY POSITIVE SELECTION OF ADENOVIRAL RECOMBINANTS THROUGH ECTOPIC EXPRESSION OF THE ADENOVIRUS PROTEASE

5

## Abstract

Disclosed is a new system for generating recombinant adenovirus vectors and adenovirus-based expression libraries, by positive selection of recombinants deleted for the endogenous protease gene, which gene is expressibly cloned into another region of the adenoviral genome. In a preferred embodiment, the invention allows positive selection of E1-deleted, protease-deleted recombinant adenovirus vectors comprising an exogenous gene or an expressible piece of exogenous DNA, by providing an expression cassette comprising the protease gene and the exogenous DNA inserted in place of E1 region in a shuttle vector. *In vivo* recombination of the shuttle vector with a protease-deleted adenoviral genome in suitable non-complementing cells generates viable recombinants only when rescuing the protease cloned in E1 region. Non-recombinant viral genomes are not able to grow due to the deletion of the protease gene, ensuring that only recombinant viral plaques are generated. This positive selection can be used for the generation of a large number of high purity recombinant adenovirus vectors and allows generation of adenovirus-based libraries with diversity exceeding  $10^6$  clones.